PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	John A. Arcadi
Application No.	:	08/516,004
Filed	:	August 16, 1995
Title	:	COMPOSITION AND METHOD FOR TREATING PROSTATE CANCER
Grp./Div.	:	1200
Examiner	:	J. Goldberg
Docket	:	28095/RWJ/H29

DECLARATION OF JOHN A. ARCADI

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Assistant Commissioner for Patents
Washington, D.C. 20231

Commissioner:

I, John A. Arcadi, declare:

1. I am the inventor named in this patent application.

2. I am the author of the Arcadi 1986 and 1990 references of record in this application, and on which the Examiner relies in rejecting all the claims in this application.

3. My 1986 article discloses the testing of a saline suspension of rhodamine-123 (Rh-123) on rats which had been implanted subcutaneously in the flanks with transplantable R3327-H Dunning rat prostate adenocarcinoma. For that test I wanted to use a solution of 5 mg. of Rh-123 per ml. of solution, but the Rh-123 was in the form of crystalline particles, which would not dissolve entirely in the saline solvent. Accordingly, I stirred the saline suspension vigorously to suspend the Rh-123 particles, filled a hypodermic syringe with the appropriate amount of saline suspension (which contained dissolved Rh-123 and undissolved particles of Rh-123 in the amount of 5 mg. of Rh-123 per ml. of suspension), and within five to ten seconds injected the suspended Rh-123 into the rat being treated. The subcutaneous injection of the saline suspension of Rh-123 described in my 1986 article would be unacceptable for treating patients because the suspension would result in uncertain dosage, and there would be an unknown amount of solubilizing of the Rh-123.

4. To overcome the problem of using Rh-123 in a saline suspension, I tried an experiment in which I dissolved the Rh-123 in a solution containing 50% dimethylsulfoxide (DMSO) in distilled water. The Rh-123 was sufficiently soluble (5 mg. Rh-123 per ml. of solvent) in the mixture of DMSO and water so as to avoid having to use a suspension. However, I later learned during the course of my work described in my 1995 article that the 50% DMSO mixture with water was unacceptable for administering Rh-123 because it killed some of the mice tested. This is referred to in my patent application on page 6 beginning at line 25 under the section entitled "Toxicity Studies on Mice". Incidentally, in reviewing the description in the application under that heading, I noticed an error in reporting my work. The sentence which begins on page 6, line 29 should read as follows:

"For each solvent there were five groups of five mice each, with the Rh-123 dose per group being 0, 2.0, 7.5, 15, and 20 mg/kg of body weight."

The following sentence should have been inserted after the one set forth above:

"A sixth group of five mice were not given any solvent or Rh-123."

The following table sets forth more fully and accurately the results of the toxicity study referred to in my patent application:

Group No.	No. of Deaths	Treatment
Group 1a	3	20 mg/kg* Rh-123 in 50% DMSO + 50% water**
Group 1b		20 mg/kg Rh-123 in 5% alcohol + 5% glucose*** in water
Group 2a	1	15 mg/kg Rh-123 in 50% DMSO + 50% water
Group 2b		15 mg/kg Rh-123 in 5% alcohol + 5% glucose in water
Group 3a		7.5 mg/kg Rh-123 in 50% DMSO + 50% water
Group 3b		7.5 mg/kg Rh-123 in 5% alcohol + 5% glucose in water
Group 4a		2.0 mg/kg Rh-123 in 50% DMSO + 50% water
Group 4b		2.0 mg/kg Rh-123 in 5% alcohol + 5% glucose in water
Group 5a	2	50% DMSO + 50% water only
Group 5b		5% alcohol + 5% glucose in water only
Group 6		None

*Mg. of Rh-123 per kg. of body weight

**Distilled water was used for all treatments

***The ethyl alcohol was present in the solution in the amount of 5% by volume, and the glucose 5% by weight

Each group in the above table included five mice. As the table shows, three of the five mice in Group 1a died when injected with a dose of 20 mg/kg of Rh-123 dissolved in a mixture of 50% DMSO and 50% distilled water at a concentration of 5 mg. Rh-123 per ml. of solution. One of the five mice in Group 2a died when injected with 15 mg/kg Rh-123 dissolved in a solution of 50% DMSO and 50% distilled water by volume at a concentration of 5 mg. of Rh-123 per ml. of solution.

Two of the five mice in Group 5a died when injected with a solution of 50% DMSO and 50% distilled water containing no Rh-123.

None of the mice died when injected with any of the ethyl alcohol/glucose solutions, even at a dose of 20 mg/kg Rh-123 in a solution of 5 mg. of Rh-123 per ml. of solution, included which 5% ethyl alcohol by volume and 5% glucose by weight in distilled water.

5. The toxicity studies set forth in paragraph 4 above show that the use of the DMSO solutions described in my 1990 article would be totally unacceptable for treating patients.

6. The prostate carcinoma treated in the rats as described in my 1990 article was induced by subcutaneous inoculation with a suspension of Pollard III cells. Thus, none of the experiments described in my 1986 or 1990 articles used Rh-123 to treat autochthonous prostate cancer, that is, prostate cancer which occurs originally in the prostate gland.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date 2/3/97

By John A. Arcadi
John A. Arcadi

RWJ/cls
AEM PAS73132.1-*-2/12/97 4:08 pm

Evaluation of Prostate-Specific Antigen as a Surrogate Marker for Response of Hormone-Refractory Prostate Cancer to Suramin Therapy

By Rajeshwari Sridhara, Mario A. Eisenberger, Victoria J. Sinibaldi, Leonard M. Reyno, and Merrill J. Egorin

Purpose: We evaluated the surrogate role of serum prostate-specific antigen (PSA) using prospectively collected information from patients with hormone-refractory prostate cancer (HRPC) treated with suramin.

Materials and Methods: Data from 103 patients were analyzed using survival analysis, exploratory analysis, and regression analysis.

Results: There was a significant survival difference between groups of patients with a PSA decrease of ≤ 0% or greater than 0% ($P = .018$). There were no significant overall survival differences between groups of patients with PSA decreases less than 50% or ≥ 50% and less than 75% or ≥ 75%. Tree-based modeling did not define a specific threshold percentage PSA change as a response criterion. For a response of 1-year survival, sensitivity increased (0.91 *v* 0.69), but specificity decreased (0.37 *v* 0.62), with a 75% versus 50% PSA decrease used as classification criterion. Differences between the area under the receiver-operating curves (ROCs) with 50% and 75% PSA decreases as threshold values were small. For a response of 1-year survival, attributable proportions were 0.38 and 0.68, respectively, with 50% and 75% PSA decreases as threshold values. When pretreatment variables were assessed by Cox proportional hazards model, hemoglobin level was the most significant predictor of survival. When percentage PSA change was included in the model, hemoglobin level remained the most significant factor, but percentage PSA change was also a weak, but statistically significant, factor. PSA was a weak, but statistically significant, predictor of survival in Cox proportional hazards model with PSA as a time-variant covariate.

Conclusion: Reduction in PSA level has weak prognostic significance with respect to survival in HRPC patients, but, currently, PSA reduction cannot be used as a reliable response criterion to evaluate treatment efficacy in individual patients. Prospective, randomized studies, including prospective measurement of other indices related to symptomatic clinical benefits, are required.

J Clin Oncol 13:2944-2953.

EVALUATION OF THE therapeutic effects of systemic treatments in prostate cancer is obscured by significant methodologic problems. Many of these difficulties are related to the disease itself. The most common metastatic site is bone, and is manifested by diffuse osteoblastic lesions that cannot be measured reliably to allow for assessments of therapeutic benefits. Serum markers, such as acid phosphatase, have not shown a good correlation with disease status, and use of unidimensional parameters (such as prostatic size) are controversial and not devoid of substantial potential bias.[1-5] Because development of new therapeutic regimens and agents must be based on evaluation of therapeutic responses, the development of useful, reliable response criteria in prostate cancer remains a subject of intense investigation.

In recent years, following the identification of prostate-specific antigen (PSA) as a prostate-specific peptide, much emphasis has been placed on defining the clinical utility of this new marker[6] in patients with metastatic prostatic carcinoma. Although PSA is not a cancer-specific peptide, major elevations in its serum concentration are usually seen in patients with extensive metastatic disease.[7] Recent experience with various androgen deprivation approaches has demonstrated that serum PSA concentrations will normalize, and frequently reduce dramatically, following initiation of treatment in the vast majority of patients with stage D_2 adenocarcinoma of the prostate.[8] In patients with hormone-refractory prostate cancer (HRPC), in whom the development of nonhormonal cytotoxic chemotherapy is the main objective, major changes in serum PSA determinations are seen less frequently and to a lesser magnitude. Over the past few years, various attempts have been made to correlate changes in serum PSA concentrations with tumor responses and disease progression in patients with HRPC.[9,10] These have primarily involved a retrospective analysis of PSA changes along with other elements of

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prognostic importance. Such analyses suggested a possible role for PSA as an appropriate surrogate marker for therapeutic effects in patients with advanced HRPC.[10]

The University of Maryland Cancer Center has conducted two phase I studies of suramin that included many patients with HRPC. The first study (study A), which was conducted between 1990 and 1993 and enrolled 69 patients with HRPC, had the following main objectives: (1) to define the overall toxicity of suramin when administered as intermittent, short (1-hour) intravenous (IV) infusions; (2) to determine the feasibility of using adaptive-control-with-feedback dosing to maintain plasma suramin concentrations within a target range; and (3) to develop a population model of suramin pharmacokinetics. The results of this study have been published elsewhere.[11,12] The second study (study B), which was conducted between 1993 and 1994 and enrolled 40 patients with HRPC, was a logical successor to the first trial. This second study was designed to characterize the variability in plasma suramin concentrations and the clinical/laboratory toxicities associated with the delivery of a pharmacokinetically based, fixed suramin-dosing regimen.[13,14]

In this report, we analyze our clinical and laboratory experience, focused on PSA, in the aforementioned studies. In both studies, one of the objectives was to assess the antitumor activity of suramin in the patients with HRPC. While therapeutic efficacy was not the major end point in these studies, each study included an extensive, prospective collection of clinical, laboratory, and radiologic data, including weekly determinations of PSA during treatment and monthly thereafter. Data from both studies were analyzed retrospectively to examine if serum PSA changes directly correlated with the length of survival and to explore the potential role of PSA changes as a biologic surrogate end point for assessing response in individual patients.

MATERIALS AND METHODS

Patients

Eligible patients in the two studies (A and B) had histologically confirmed HRPC, Karnofsky performance status (PS) ≥ 60%, one or more prior chemotherapeutic regimens, evidence of progression on prior treatment, adequate renal function, adequate hepatic function, adequate bone marrow reserve, normal coagulation studies, and the ability to understand and sign an approved informed consent form.[11,13,14] Patients were not concurrently receiving other specific anticancer drugs except luteinizing hormone-releasing hormone (LHRH) analogs, which were continued to maintain plasma testosterone within the castrate range (< 50 ng/dL) in those patients with no prior orchiectomy. Other hormones (except hydrocortisone, see later) were discontinued before study entry.[11,13,14] The analysis reported here excluded patients with pretreatment PSA concentrations less than 4 ng/mL.

Therapy

All patients received 1-hour infusions of suramin at doses and schedules designed to maintain plasma suramin concentrations between 100 and 300 μg/mL throughout the study period until dose-limiting toxicity or disease progression.[11-14] In addition to suramin, patients received subcutaneous injections of vitamin K (10 mg/wk) and oral hydrocortisone (40 mg/d).

Data

The following clinical and laboratory data were collected before treatment: age, performance status (PS), sites of metastases (visceral, bone, and nodal), exposure to flutamide before suramin, hemoglobin level, platelet count; creatinine clearance, serum albumin level, alkaline phosphatase level, calcium level, potassium level, magnesium level, lipase level, lactate dehydrogenase (LDH) level, acid phosphatase level, and PSA concentration. In addition, at every clinic visit during (approximately weekly) and after treatment (approximately monthly), serum PSA and acid phosphatase levels were measured.

Statistical Methods

All survival analysis procedures were conducted using SAS Software (SAS Institute Inc, Cary, NC) and the plots were obtained using EGRET software (Statistics and Epidemiology Research Corp and Graphics Software Systems, Cambridge, MA). The Kaplan-Meier method[15] was used to estimate the overall median survival with Greenwood confidence limits of the study patients. To identify the relationship between survival and changes in serum PSA concentrations, univariate survival analyses were conducted using Kaplan-Meier estimation and the log-rank test.[16]

The following analyses were further conducted to evaluate PSA changes as a surrogate end point for survival. In these analyses, PSA changes at 4 weeks (24 to 32 days) from baseline (pretreatment) levels were examined initially. In this regard, a landmark approach was used in selecting PSA change at 4 weeks instead of PSA nadir values so as to avoid the bias that would exist when length of survival itself influences the chance of a patient being classified into one group or the other.[17]

First, tree-based modeling[18] was used to identify a threshold level of percentage reduction in serum PSA concentration. In this procedure, a survival time of 1 year was used as the decision criterion (estimated survival of HRPC patients based on historical data). Using S-plus for Windows software (Statistical Sciences Inc, Seattle, WA), the model was fitted by binary, recursive partitioning whereby a data set was successively split into increasingly homogeneous subsets until it was not feasible to continue. This was done by repeated sequential examination of all possible partitions to find the variable value that best predicted the outcome.

Second, sensitivity analyses[19] were conducted for arbitrarily defined specific threshold levels of percentage reduction (50% and 75%) in PSA level from baseline to 4 weeks of therapy (ΔPSA), and survival times of 1 and 2 years to assess the sensitivity, specificity, and false-negative and false-positive rates. For the purpose of these analyses, the sensitivity, specificity, and false-negative and false-positive rates were defined as follows: sensitivity = (no. of patients with ΔPSA < x% and survival < y years)/(no. of patients with survival < y years); specificity = (no. of patients with ΔPSA ≥ x% and survival ≥ y years)/(no. of patients with survival ≥ y years); false-negative rate = (no. of patients with ΔPSA ≥ x% and survival < y years)/(no. of patients with ΔPSA ≥ x%); false-positive

rate = (no. of patients with $\Delta PSA < x\%$ and survival $\geq y$ years)/(no. of patients with $\Delta PSA < x\%$).

Third, to examine further the difference in the predictive power between the two threshold values (50% and 75% reduction in serum PSA), the area under the receiver-operating characteristic curve (ROC)[20] was computed using STATA 3.1 software (Stata Corp. College Station, TX). A model with no predictive power has an area of 0.5, and a perfect model (sensitivity and specificity = 1) has an area of 1. Further, to quantify the marker-survival association, attributable proportion (AP)[21] was computed. An AP of 1 implies perfect association and that the marker can be used as an intermediate end point. An AP of 0 implies no association and that the marker is not a surrogate of survival.

Fourth, further univariate analyses were conducted using Kaplan-Meier estimation and log-rank tests[16] to identify any confounding baseline variables that could influence survival. The pretreatment variables considered included performance status, age, treatment cohort, hemoglobin level, platelet count, serum alkaline phosphatase level, serum albumin level, LDH level, acid phosphatase level, PSA concentration, creatinine clearance, and prior exposure to flutamide. In this step of the analyses, the continuous variables such as age, hemoglobin level, platelet count, serum alkaline phosphatase level, serum albumin level, LDH level, acid phosphatase level, serum PSA concentration, and creatinine clearance were categorized into two groups with the respective median values as the cut-off values, in the absence of any other prognostically established cut-off values. The significant prognostic factors identified by the univariate analyses were then fitted by Cox proportional hazards model.[22]

Fifth, in addition, to examine if the serial serum PSA concentrations measured over a period of time were related to survival, the PSA measurements were treated as a time-variant factor, and a time-variant Cox hazards model[22] was fit to the survival data.

Although patients were not randomly chosen, the previously stated hypotheses-testing procedures were conducted, based on the assumption that the study patients constituted a representative group of the population of patients with HRPC. Also it was assumed that the study population represented a homogeneously selected group, who fulfilled predefined eligibility criteria, received the same treatment, and were evaluated and monitored in the same fashion. We recognized that the results of these analyses should not be extended to the population at large, but could be used to generate valid hypotheses that might be tested further by prospective, randomized studies.

RESULTS

Data were analyzed from a total of 103 assessable patients of the 109 patients with HRPC who were treated with suramin. Among six patients considered nonassessable for this study, three could not be assessed for suramin response, two were non-PSA producers, and, based on a retrospective assessment, one clearly responded to flutamide withdrawal before suramin treatment. Demographic data are listed in Table 1. Based on an overall survival analysis conducted using the Kaplan-Meier method of estimation, the median survival time in this group of 103 patients was 21 months (95% Greenwood confidence interval, 15 to 27 months) (Fig 1).

Thirteen patients had an increase in serum PSA concentration from baseline measurements at 4 weeks. Fourteen

Table 1. Patient Characteristic

Characteristic		No. of Patients
Total assessable*		103
Target plasma suramin concentration		
Cohort 1 (175-300 μg/mL, study A[11])		22
Cohort 2 (150-250 μg/mL, study A[11])		23
Cohort 3 (100-200 μg/mL, study A[11])		22
Cohort 4 (fixed regimen, study B[13,14])		36
Age, years		
Median	66	
Range	44-85	
PS		
0		33
1		61
2		9
Pretreatment PSA (ng/mL)		
4-10		2
11-20		7
21-50		13
51-100		19
101-200		17
201-300		15
301-400		6
401-500		7
501-1,000		7
1,001-2,000		7
> 2,000		3
Pretreatment PSA (ng/mL)		
Median	176	
Range	4-4,737	
HRPC		103
Extent of disease		
Bone only		78
Bone and visceral (lung liver)		9
Bone and soft tissue		15
Soft tissue only		1
Prior treatment		
Local treatment		
Prostatectomy		12
Radiation		28
Both		2
Implants		3
None		58
Prior palliative radiation		63
Prior systemic treatment		
First-line endocrine treatment		103
Orchiectomy/GnRH + flutamide		43
Orchiectomy/GnRH alone		50
Estrogens/other		10
Second-line treatment		59
Flutamide		34
Megestrol acetate		5
Ketoconazole (+ 1-hydrocortisone)		5
Glucocorticoids		11
Orchiectomy		2
Orchiectomy/GnRH + flutamide		2
Third-line hormonal manipulation		18
Flutamide		2
Estrogens		2
Megestrol acetate		4
Aminoglutethimide + hydrocortisone		5
Ketoconazole + hydrocortisone		5
No. of prior endocrine treatments		
Median	2	
Range	1-3	
Time from start of first endocrine manipulation to suramin (days)		
Median	690	
Range	95-5,875	
Prior chemotherapy		18

Abbreviation: GnRH, gonadotropin-releasing hormone.
*One hundred three of 109 assessable for this analysis.

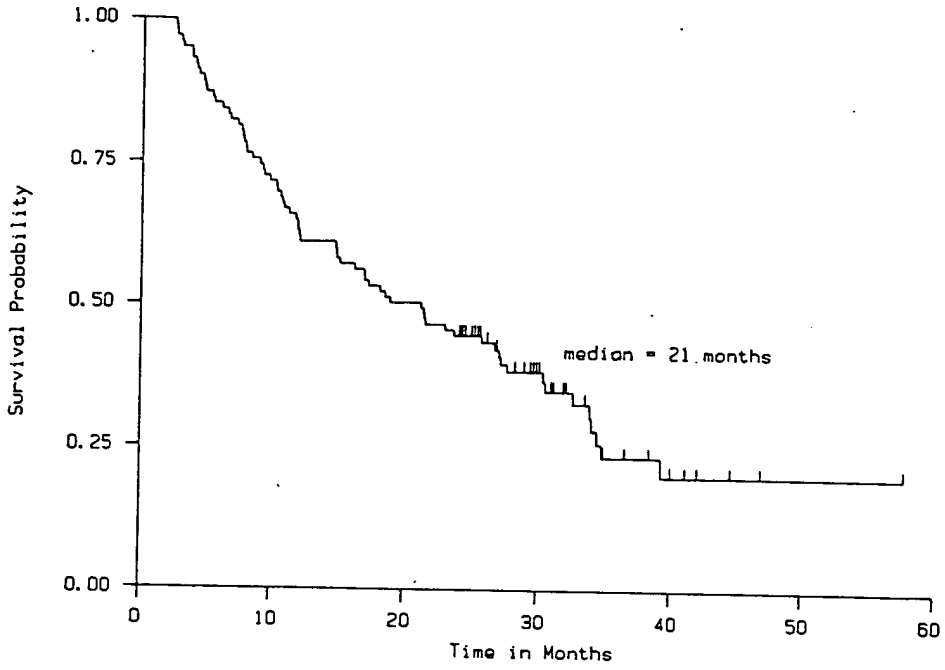

Fig 1. Overall survival curve (n = 103).

patients had a reduction in serum PSA concentration of 0% to 24.9% from baseline measurement at 4 weeks, 21 patients had a 25% to 49.9% reduction, 24 had a 50% to 74.9% reduction, and 26 had a 75% to 100% reduction. Five of 103 patients did not have PSA concentrations available at 4 weeks. There was a statistically significant difference between the survival curve of the group of patients whose serum PSA concentration increased and that of the group of patients who had any reduction in serum PSA concentration after 4 weeks of treatment (Fig 2A) (P = .018). No significant difference was observed between the overall survival curves (Fig 2B) of the group of patients who had a ≥ 50% reduction in serum PSA concentration and that of the group of patients who had a less than 50% reduction (P = .072). Similarly, no significant difference was observed between the estimated overall survival curves (Fig 2C) of groups of patients who did or did not achieve a 75% reduction in serum PSA concentration at 4 weeks after the start of suramin therapy (P = .113). In each of these comparisons, a greater percentage decrease in PSA was associated with longer survival in the initial 2 years of follow-up evaluation (Wilcoxon test, $P < .04$).

Thus, to identify a specific threshold value of serum PSA or percentage reduction in serum PSA that would allow classification of risk groups, a number of additional exploratory analyses were conducted. As a first-step, a tree-based model was fit to the data, using survival of 1 year as the decision criteria. In this analysis, the split-on-start partitioned 98 observations (PSA at 4 weeks was not available in five of 103 patients) into groups of seven (< −15.1% reduction in PSA) and 91 (≥ −15.1% reduction in PSA) observations with probabilities of survival of less than 1 year of 1.0 and 0.3, respectively. The group of 91 observations was further split into groups of 60 (< 70.9% reduction in PSA) and 31 (≥ 70.9% reduction in PSA) observations with probabilities of survival of less than 1 year of 0.4 and 0.1, respectively. This procedure of partitioning continued yielding probabilities of survival of less than 1 year ranging from 0.0 to 1.0. As the partitioning continues to develop, the numbers in individual subgroups decreases. Thus, the individual estimated probabilities become less reliable. Also in the first split, the deviance, which is the measure of node heterogeneity (a deviance of zero corresponds to a perfectly homogeneous node), was 127. The misclassification error rate in this classification tree was 0.20 without pruning of the tree, and 0.28 with pruning of the tree.

These results of the tree-based model suggested the possibility of three groups of patients based on percentage reduction in PSA at 4 weeks. These groups were as follows: (1) a poor-prognosis group with a more than 15% increase of PSA (n = 7; probability of survival of < 1 year, 1); (2) a good-prognosis group with a greater than 70% decrease of PSA (n = 31; probability of survival of < 1 year, 0.1); and (3) an unknown-prognosis group (n = 60; probability of survival of < 1 year, 0.4). On further comparison of survival in these three groups, a significant

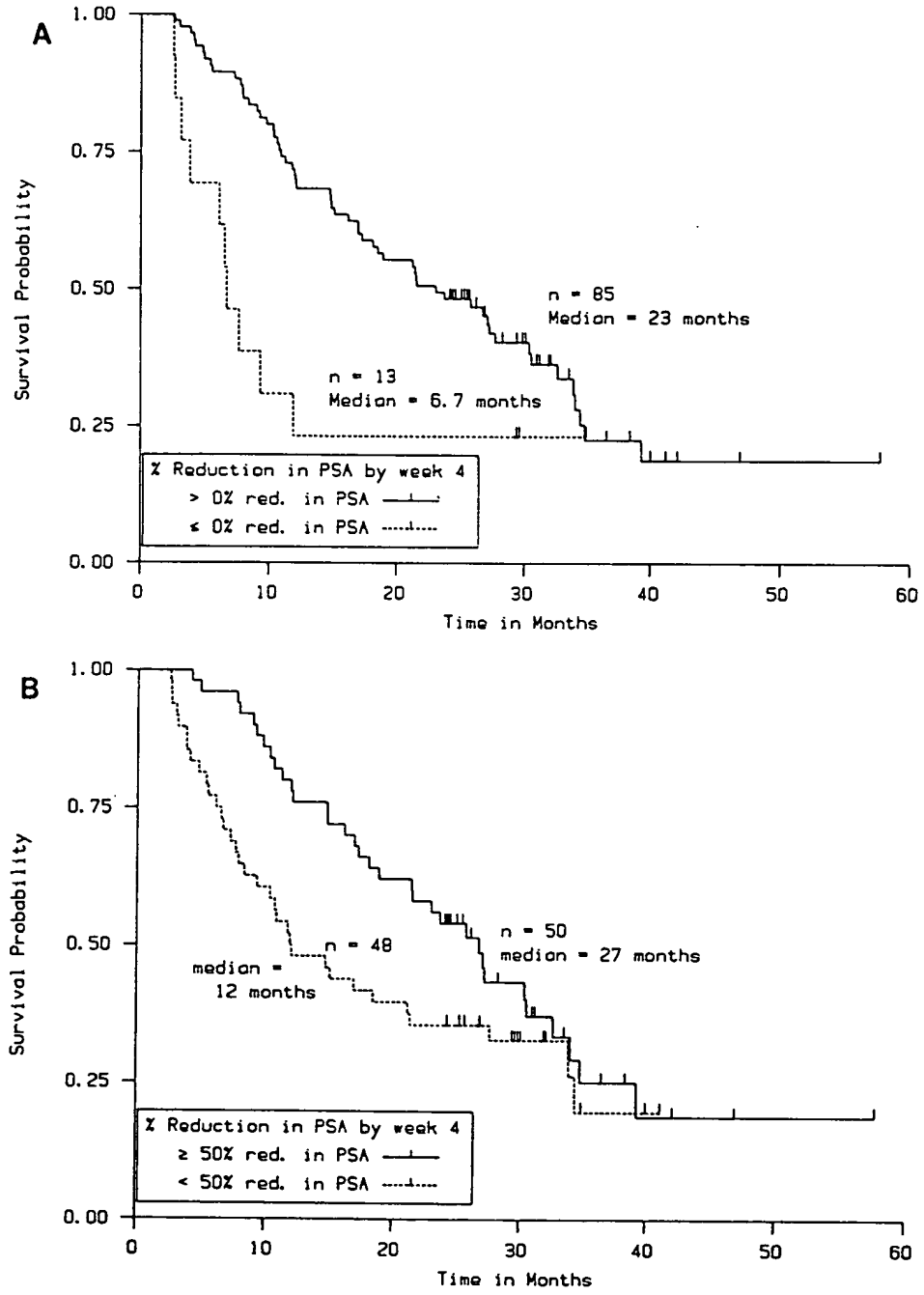

Fig 2. Survival curves for the groups with percentage reduction in PSA, from the start of therapy to 4 weeks of therapy, of (A) ≤ 0% (---) and > 0% (——); (B) < 50% (---) and ≥ 50% (——). (Cont'd on next page.)

difference was observed (P = .0001, log-rank test) (Fig 3). However when groups 2 and 3 were compared separately, no significant difference was observed (P = .166). Also it is to be noted that there are only seven observations in group 1. Hence, the classification of prognostic groups needs to be further tested, preferably within the context of a prospective, randomized study with sufficient numbers to detect meaningful differences.

In view of the previous observations, we felt that a sensitivity analysis was necessary. The results of the sensitivity analyses with arbitrarily chosen threshold values of 50% and 75% reduction in PSA concentrations after 4 weeks of treatment are listed in Table 2. It should be noted that although the sensitivity increases with 75% reduction in PSA as the threshold value, the specificity decreases substantially.

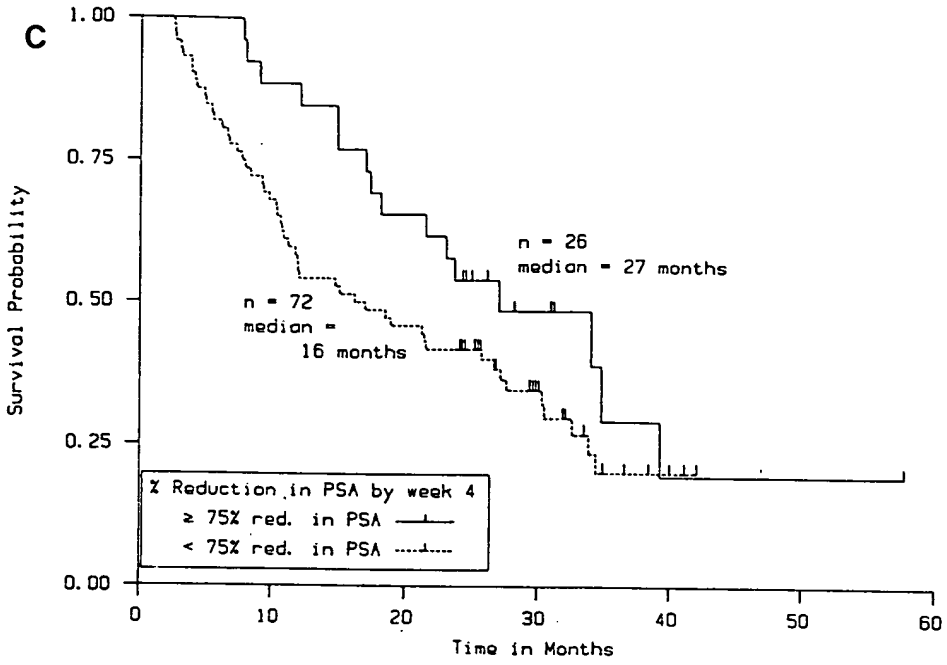

Fig 2 (cont'd). (C) < 75% (---) and ≥ 75% (——). Total N = 98 patients; PSA was not measured at 4 weeks in 5 patients.

These results were further examined by computing the average of sensitivity and specificity or the area under the ROC. With 1-year survival as the decision criteria, the area under the ROC with threshold value of 50% reduction in serum PSA was marginally greater when compared with the area with 75% reduction in serum PSA as the threshold value (0.65 v 0.64). With 2 years as the decision criteria, the areas under the ROC with the same threshold values of 50% reduction in PSA versus 75% reduction in PSA were 0.59 versus 0.55, respectively.

In addition to these analyses to quantify the marker-survival association, AP values, as described by Schatzkin et al.[21] were computed. With 1-year survival as the deci-

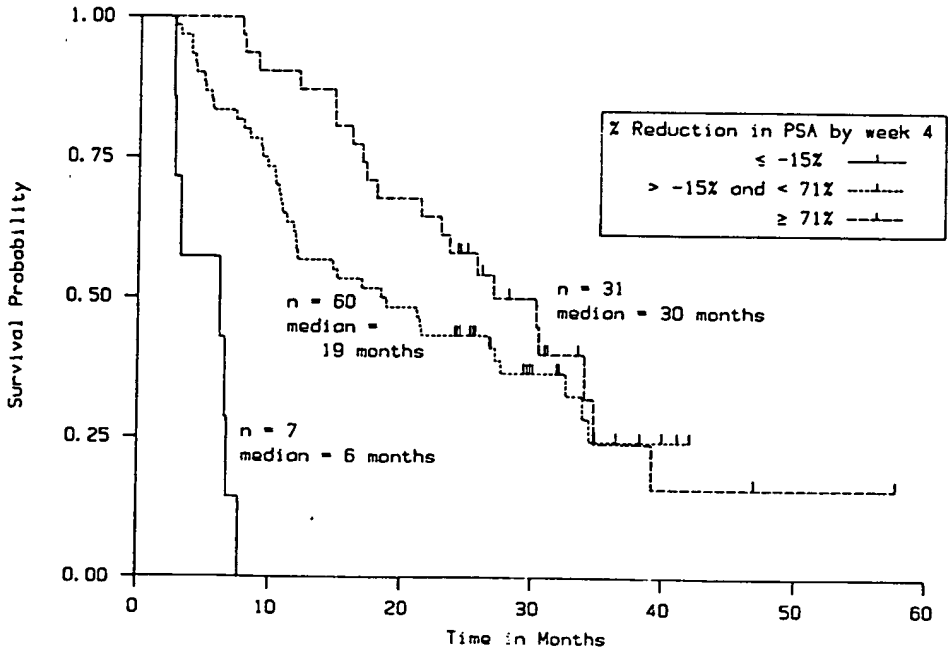

Fig 3. Survival curves for the groups with percentage reduction in PSA, from the start of therapy to 4 weeks of therapy, of ≤ −15% (——), −15% −71% (---), and ≥ 71% (– –). Total N = 98 patients; PSA was not measured at 4 weeks in 5 patients.

Table 2. Results of Sensitivity Analyses

PSA Reduction Threshold (%)	Survival Criterion (years)	Sensitivity	Specificity	False-Negative Rate	False-Positive Rate
50	1	0.69	0.62	0.22	0.50
50	2	0.70	0.50	0.46	0.35
75	1	0.91	0.37	0.12	0.56
75	2	0.78	0.32	0.46	0.42

NOTE. N = 98 patients; PSA was not measured at 4 weeks in 5 patients.

sion criteria, the AP values were 0.38 and 0.68, respectively, with 50% and 75% reduction in PSA as threshold values. However, with 2-year survival as the decision criteria, these AP values were 0.20 and 0.16, respectively, with 50% and 75% reduction in PSA as threshold values.

Univariate analyses relating survival and pretreatment prognostic factors were conducted to eliminate any confounding factors affecting the survival. These results are listed in Table 3. Hemoglobin level, serum albumin level, LDH level, PS, treatment cohort, baseline PSA concentration, and baseline acid phosphatase level were found to be significant factors at P less than .2. These factors were further analyzed by a multivariate analysis using Cox proportional hazards model and backward elimination procedure, treating these factors as categories as defined in Table 3, as well as using their actual measured values (continuous variables). The results of these analyses are presented in Tables 4 and 5. In the model with categoric variables (Table 4), hemoglobin, LDH, and serum albumin level were significant factors, with the group of patients with pretreatment hemoglobin levels ≤ 12.1 g/dL having two times the risk compared with the group with hemoglobin levels more than 12.1 g/dL. However, in the model with continuous variables (Table 5), PS of 2, hemoglobin level, platelet count, and baseline PSA concentration were found to be significant factors, with hemoglobin level having a risk ratio of 0.74. It should be noted that there were only nine of 103 patients with a PS of 2.

This multivariate analysis was repeated by including the percentage change in PSA at 4 weeks from the start of the therapy as another covariate. A model identical to the model presented in Table 4 was obtained in both the cases when the percentage change in PSA from baseline to 4 weeks after the start of therapy was dichotomized at 50% and 75% reduction in PSA.

When the regression analysis was conducted using the actual measurements of the variables hemoglobin level, platelet count, baseline PSA concentration, and percentage change in PSA were found to be significant, although the risk ratio was extremely close to 1 for platelet count, baseline PSA, and percentage change in PSA (Table 6).

These results led to an analysis of PSA values as a function of time, rather than at a specific point in time, such as at 4 weeks, as well as the actual measured concentrations rather than percentage change from baseline measurement, as considered earlier. This was accomplished by conducting a regression analysis using a time-variant Cox hazard model with PSA concentrations measured over a period of time as a time-variant predictor. Although the regression coefficient of PSA by this analysis was

Table 3. Univariate Analysis: Log-Rank Tests for Potential Prognostic Variables for Survival

Variable	Group*	Sample Size	No. Dead	Median Survival (Days)	P
All patients		103	70	645	
Hemoglobin level	≤ 12.1 g/dL	52	41	335	.0043
	> 12.1 g/dL	51	29	827	
Cohort target plasma concentration	175-300 μg/mL	22	21	352	.0120
	150-250 μg/mL	23	17	368	
	100-200 μg/mL	22	17	648	
	Fixed regimen	36	15	NR	
LDH level	≤ 604 U	52	31	928	.0152
	> 604 U	51	39	451	
Serum albumin level	> 4 mg/dL	46	35	409	.0160
	≥ 4 mg/dL	57	35	700	
Baseline PSA	≤ 176 ng/mL	52	32	823	.0494
	> 176 ng/mL	51	38	452	
PS	0	33	27	655	.1090
	1	61	28	813	
	2	9	7	255	
Acid phosphatase	≥ 11 U	52	34	813	.1223
	> 11 U	51	36	451	
Prior flutamide treatment†	Never	22	16	771	.3301
	First-line therapy	41	24	783	
	Second-line therapy	36	28	488	
Creatinine clearance	≤ 87 mL/min	52	36	648	.3376
	> 87 mL/min	51	34	574	
Alkaline phosphatase level	≤ 203 U	52	34	714	.3494
	> 203 U	51	36	492	
Age	≤ 66 years	54	39	472	.3870
	> 66 years	49	31	655	
Platelet count	≤ 264 /μL	53	36	645	.9300
	> 264 /μL	50	34	631	
Time of withdrawal† of flutamide before suramin	Never flutamide	22	16	771	.9937
	> 3 months	19	13	645	
	≤ 3 months	58	39	568	

Abbreviation: NR, not reached.

*Values selected represent the ≤ and > median values for this patient population.

†Information about prior flutamide treatment was not available in 4 of 103 patients.

Table 4. Results of Analysis of Maximum Likelihood Estimates Using Cox Proportional Hazards Model With Covariates (pretreatment factors) Classified Into Categories

Variable	df	Parameter Estimate	Standard Error	P	Risk Ratio	95% Confidence Interval
Hemoglobin (≤ 12.1, > 12.1 g/dL)	1	0.7529	0.2587	.0036	2.123	1.279-3.525
LDH (> 604, ≤ 604 U)	1	0.5961	0.2577	.0207	1.815	1.095-3.008
Serum albumin (< 4, ≥ 4 mg/dL)	1	0.6189	0.2593	.0170	1.857	1.117-3.087

statistically significant, the risk ratio was essentially 1 (Table 7), ie, the risk of death increased marginally with every unit increase in PSA concentration.

In 18 of 70 patients who died, data on PSA measurement within 1 week before death were available. At death, four of 18 patients had a serum PSA level between 101 and 200 ng/mL, three of 18 between 201 and 300 ng/mL, four of 18 between 301 and 400 ng/mL, one of 18 between 401 and 500 ng/mL, and six of 18 more than 500 ng/mL. The Spearman-rank correlation coefficient between survival days and PSA at death in these 18 patients was 0.62. However, in five of 18 patients, PSA concentration at death was less than the baseline PSA.

DISCUSSION

The main objective of this study was to evaluate reduction in serum PSA concentrations as a possible surrogate end point for response in HRPC. The identification of surrogate end points for clinical trials has become a major research effort in an attempt to control the cost and completion time of clinical trials.[23,24] Among the advantages of using surrogate end points to evaluate responses to treatments are that studies can be designed with smaller sample sizes and that therapeutic efficacy can be evaluated within a short time.[23-27] While surrogate end points are usually proposed on the basis of biologic rationale, there must be a demonstration of a statistically significant correlation with other established end points.[24]

The most commonly used criterion to evaluate cancer therapies is tumor shrinkage.[24] However, in the current study, most patients (81 of 103) did not present with any clinical or radiologic evidence of bidimensionally measurable disease that would allow quantitative measurement of tumor shrinkage as an indicator of response to treatment. This lack of reliable indicator parameters for response is a common occurrence in HRPC patients. Over the past few years, percentage change in serum PSA concentrations has been proposed and, in fact, commonly used as a surrogate end point to describe responses in these patients.

Based on 15 of 50 HRPC patients who achieved a ≥ 75% decline in PSA, Thibault et al[25] have reported that a 75% decline in PSA predicts survival. On the other hand, Kelly et al[10] have claimed that a 50% decline in PSA is a better predictor of survival, based on 15 of 100 HRPC patients from seven sequential protocols who achieved a ≥ 50% decrease in PSA and four of 100 patients who achieved a ≥ 80% decrease in PSA. In our first step in evaluating change in PSA concentrations as a surrogate end point, survival curves (Fig 2A, B, and C) were examined. These survival analyses suggested that any reduction in PSA was associated with better survival compared with increase in PSA. Because these results did not convincingly define a specific threshold value of PSA decline to be used in future studies for evaluating response to therapy, we conducted detailed exploratory analyses to define a specific response criterion based on PSA changes. In these analyses, two primary end points for evaluation were considered: (1) 1-year survival, and (2) 2-year survival. These arbitrary end points were se-

Table 5. Results of Analysis of Maximum Likelihood Estimates Using Cox Proportional Hazards Model With Covariates That Are Pretreatment Factors Measured as Continuous Variables

Variable	df	Parameter Estimate	Standard Error	P	Risk Ratio	95% Confidence Interval
PS = 2	1	0.9228	0.4478	.0393	2.516	1.046-6.052
Hemoglobin (g/dL)	1	−0.3084	0.0918	.0008	0.735	0.614-0.879
Platelet counts (μL^{-1})	1	−0.0036	0.0016	.0250	0.996	0.993-1.000
Baseline PSA (ng/mL)	1	0.0005	0.0002	.0037	1.000	1.000-1.001

Table 6. Results of Analysis of Maximum Likelihood Estimates Using Cox Proportional Hazards Model With Pretreatment Covariates and Percent Change in PSA From Baseline to 4 Weeks From Start of Therapy Measured as Continuous Variables

Variable	df	Parameter Estimate	Standard Error	P	Risk Ratio	95% Confidence Interval
Hemoglobin (g/dL)	1	−0.3162	0.0938	.0007	0.729	0.607-0.876
Platelet counts (μL^{-1})	1	−0.0040	0.0016	.0099	0.996	0.993-0.999
Baseline PSA (ng/mL)	1	0.0005	0.0002	.0037	1.000	1.000-1.001
% Change in PSA	1	−0.0131	0.0034	.0001	0.987	0.980-0.994

Table 7. Results of Analysis of Maximum Likelihood Estimates Using Cox Proportional Hazards Model With Time-Variant Covariate

Variable	df	Parameter Estimate	Standard Error	P	Risk Ratio	95% Confidence Interval
PSA (ng/mL)	1	0.0006	0.0001	.0001	1.001	1.000-1.001

lected because the median survival time in the historical group of HRPC patients is typically less than 1 year,[5] and the median survival time in the group of patients considered in this study was almost 2 years.

The partitioning method for classification and decision making of tree-based modeling[18] was unable to exhibit a decisive threshold value of change in PSA concentration that would classify the data into two groups, with survival times of less than 1 year and ≥ 1 year. Furthermore, subsequent sensitivity analyses indicated that although a 75% decrease in PSA concentration increased the sensitivity (0.9 and 0.8) in predicting 1- and 2-year survival, the specificity decreased to less than 0.5. In comparison, a 50% decrease in PSA concentration had a better balance between sensitivity and specificity (both ≥ 0.5) (Table 2). Therefore, overall error rates with a 50% decrease in PSA as the response criterion are likely to be less than with a 75% decrease in PSA as the response criterion. Also, the area under the ROC with a 50% decrease in PSA as the predictive criterion was larger, although marginally, than the area under the ROC with a 75% decrease as the predictive criteria. Thus, similar to the aforementioned sensitivity analyses, these results also indicated that a 50% decrease in PSA was comparatively a better predictor of response than was a 75% decrease in PSA.

However, the attempt to validate 50% and 75% decreases in PSA concentrations as surrogate end points of response (1-year survival) by computing attributable proportions[21] suggested that the response was more attributable to a 75% decrease in PSA rather than 50% decrease. However, with a surrogate end point of response of 2-year survival, the attributable proportions were small for both 50% and 75% decreases in PSA. These results prevent us from hypothesizing in a more conclusive manner which PSA decline is more appropriate for use as a response indicator. In addition, this raises the issue of whether a 50% decrease in PSA or a 75% decrease in PSA at 4 weeks after therapy is a valid surrogate end point of the chosen response of 1- or 2-year survival.

One reason for this apparent anomaly could be that several other confounding pretreatment factors could also be influencing survival. Thus, as a first step, univariate analyses using Kaplan-Meier survival estimates and log-rank tests were conducted to identify significant prognostic factors. It should be noted that in this step the continuous variables, such as age, hemoglobin level, platelet count, serum alkaline phosphatase level, serum albumin level, LDH level, acid phosphatase level, serum PSA concentrations, and creatinine clearance, were categorized into two groups with the respective median values as the cut-off values. A choice of different cut-off values might well produce a different set of results; however, a cut-off value more rational than the median value was not obvious.

Multivariate analyses were conducted, using Cox proportional hazards model and a backward elimination procedure, to find the most significant predictors of survival. The results of these analyses in these patients with HRPC, suggest the following: (1) pretreatment hemoglobin level is a significant predictor of survival in all of the models considered; and (2) pretreatment PSA concentration, and percentage change in PSA from baseline to the measurement at 4 weeks from the start of therapy, are weak, significant predictors of survival.

A second reason for the previously stated anomaly could be that PSA exhibits a variable relationship with extent of disease.[29,30] Also, the change in PSA concentration in itself may not be a marker of survival, whereas the actual PSA measurement at a given time may be related to survival. Thus, to establish a relationship between risk of death and the monitoring of PSA concentrations, a Cox proportional hazards model with PSA concentrations as a time-dependent covariate was used. These results indicate that the risk of death does not alter much (risk ratio, ~ 1) with every unit increase in PSA concentration, and that PSA concentration is a significant predictor of survival.

Finally, it is not known whether suramin treatment improves survival in men with HRPC, and this may be an additional reason why reduction in PSA in individual patients fails to predict for improved survival in the current study. While survival is a reliable objective end point, it is possible that the reduction in PSA may be more closely related to other, more palliative end points, which may reflect a more realistic end point for patients with HRPC. In this regard, indices of quality of life, pain control, and changes in PS could represent possible measures of therapeutic efficacy in this disease, which is typically characterized by the lack of bidimensionally measurable lesions. The phase I/II protocols on which the current patients were treated were not designed to evaluate these parameters rigorously and prospectively. Whether this or any other PSA model will reflect improvements in these indices would be of considerable interest. This is particularly important as the measurement

of these indices is both cumbersome and expensive, yet they have been proposed and used as end points in randomized clinical trials for patients with HRPC.

In summary, this study demonstrates that reduction in PSA measurements cannot be used as a reliable response criterion to evaluate treatment effects in individual patients with HRPC, although any reduction in PSA may be associated with improved survival. This hypothesis should be tested further in randomized studies in which patients are stratified by pretreatment hemoglobin levels. Ideally, such studies should also prospectively measure other indices related to symptomatic clinical benefits, so that the relationship of PSA changes to these other clinically relevant end points can be pursued.

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